(3)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

DATE: **16 October 2007**

SUBJECT: **Clothianidin. Tolerance Petition Requesting the Establishment of Permanent Tolerances (Associated with Section 3 Registration) for Food/Feed Use of the Insecticide as a Seed Treatment on Sugar Beets. Resolution of Rotational Crop Data Deficiency. Summary of Analytical Chemistry and Residue Data.**

Petition Number:	**6F7159**
PC Code:	**044309**
DP Numbers:	**335355 (Summary Document)**
	330010 (Rotational Crop Data Requirement)
Decision Numbers:	**372786 (Summary Document)**
	367524 (Rotational Crop Data Requirement)
Regulatory Citation:	**40CFR §180.586**
Chemical Class:	**Neonicotinoid (Chloronicotinyl) Insecticide**
Trade Name:	**Poncho Beta**
MRID Numbers:	**47007809, 47007810, 46826901**

FROM: William T. Drew, Chemist
Registration Action Branch 2, Health Effects Division (7509P)

THROUGH: Douglas Dotson (PhD), Chemist
Michael A. Doherty (PhD), Senior Scientist
Registration Action Branch 2, Health Effects Division (7509P)

TO: Kable Davis/Venus Eagle, RM Team 1
Julie Chao/John Hebert, RM Team 7
Insecticide Branch, Registration Division (7505P)

This Residue Chemistry Summary Document was originally prepared under contract by Dynamac Corporation (1910 Sedwick Road, Building 100, Suite B; Durham NC 27713). The document has been reviewed by the Health Effects Division (HED), and revised to reflect current Office of Pesticide Programs (OPP) policies.

Received in
RRC 10/29/2007
CW

Executive Summary

Clothianidin (with CAS Registry Number 210880-92-5, and CAS Name [*C(E)*]-*N*-[(2-Chloro-5-thiazolyl) methyl]-*N'*-methyl-*N''*-nitroguanidine) is a systemic neonicotinoid insecticide (chloronicotinyl class) currently registered for use as a seed treatment on corn, canola (rapeseed), and sorghum, as well as for foliar applications to pome fruits, grapes, and potatoes. Clothianidin is the major metabolite of thiamethoxam. Permanent tolerances are established for residues of clothianidin in or on plant commodities at 0.01-1.0 ppm, and in milk at 0.01 ppm (40CFR §180.586[a]), while time-limited tolerances are established for clothianidin in sugar beet roots and tops at 0.02 ppm (40CFR §180.586[b]). Tolerances are also established for the indirect residues of clothianidin at 0.02 ppm in nongrass livestock feeds; forage, fodder, and straw of cereal grains; forage, fodder, and hay of grasses; and soybean forage and hay (40CFR §180.586[d]).

Bayer CropScience (Bayer) has submitted a petition (PP#6F7159) proposing the use of a multiple active ingredient (MAI) formulation containing 3.33 pounds per gallon (lb/gal) of clothianidin, and 0.44 lb/gal of beta-cyfluthrin (Poncho Beta, with EPA File Symbol 264-RNLA) as a seed treatment for sugar beets (cyfluthrin is not addressed in this document). This product is formulated as a suspo-emulsion (SE), which is a combination formulation consisting of a suspension concentrate coupled with an oil-based emulsion. The proposed use is restricted to commercial seed treaters utilizing commercially available equipment designed for seed treatment only (liquid or slurry treaters). Applications by agricultural establishments using on-farm equipment for treating seeds at planting are prohibited. For clothianidin, the proposed use rate is 0.132 lb active ingredient (ai) per 100,000 seeds, which is equivalent to 0.068-0.095 lb ai per acre (lb ai/A), based on typical planting rates of 53,000-72,000 seeds per acre. In conjunction with this use, Bayer is proposing permanent tolerances for clothianidin, as listed below.

Beet, sugar, roots	0.02 ppm
Beet, sugar, tops	0.04 ppm
Beet, sugar, molasses	0.06 ppm

The nature of the residue in plants has been adequately delineated, based on acceptable corn, sugar beet, apple, and tomato metabolism studies. HED has determined that the parent compound is the only residue of concern (ROC) in primary crops for both tolerance expression and risk assessment purposes (D282449, Yan Donovan, 4/25/2003). However, for new uses on root crops and/or leafy vegetables, samples should also be analyzed for residues of TMG. Additionally, for rotational crop field trial studies, samples should be analyzed for parent, TZNG, and (if possible) MNG. The nature of the residue in livestock is also understood, based on acceptable goat and hen metabolism studies. For risk assessment, the ROCs in ruminants include parent and the metabolites TZU, TZG, TZNG, and ATMG-Pyruvate, while the ROCs in poultry include parent and the metabolites TZU, TZG, TZNG, and ATG-Acetate. However, only parent needs to be included in the tolerance expression for livestock commodities.

Adequate LC/MS/MS methods are available for both collecting data, and enforcing tolerances for clothianidin residues in plant commodities (Bayer Methods 00552, 00552-M001, and 109240-1), and livestock commodities (Bayer Method 00624). The validated limit of

quantitation (LOQ) for clothianidin in plant commodities is 0.010 ppm, except in wheat straw (0.020 ppm), while the validated LOQs are 0.010 ppm in milk, and 0.020 ppm in livestock tissues.

In the current sugar beet field trials and processing studies, residues of clothianidin and its metabolite, TMG, were determined in each commodity using another LC/MS/MS method (Bayer Method TI-002-P05-001). This method is similar to enforcement method 00552-M001, but also determines residues of TMG. For this method, residues are extracted sequentially with acetonitrile (ACN), and ACN/water, then fortified with [$^2H_6$]-internal standards of clothianidin and TMG. Residues are then acidified, purified using a C_{18} solid-phase extraction (SPE) cartridge, and determined by LC/MS/MS. Residues of clothianidin and TMG are expressed in clothianidin equivalents. The method was adequately validated in conjunction with the analysis of study samples. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots, tops, and processed fractions, with the exception of clothianidin in molasses (0.020 ppm). The statistically calculated limits of detection (LODs) for clothianidin are 0.003 ppm in roots, tops, refined sugar, and dried pulp, and 0.011 ppm in molasses. The statistically calculated LODs for TMG are 0.001 ppm in roots, 0.002 ppm in refined sugar and dried pulp, 0.003 ppm in tops, and 0.005 ppm in molasses.

Adequate storage stability data are available indicating that clothianidin is stable at -18°C for intervals of up to 24 months in sugar beet roots, corn (grain, forage, and straw), and canola seed, and for intervals of up to 6 months in potatoes. Preliminary data are also available from an on-going storage stability study indicating that TMG is stable at -15°C or colder in sugar beet leaves, potato tubers, and potato flakes and chips for intervals of up to 12 months. Provided that the storage stability study on TMG is submitted, and deemed adequate by the Agency, these data will support the current sugar beet field trials and processing study, as tops and roots were stored frozen for less than 13 months, while processed fractions were stored frozen for less than 1 month to 4.2 months.

The available sugar beet field trial data are adequate, and support the proposed seed treatment use. Following a seed treatment with clothianidin (SE) at a rate of 0.132 lb ai/100,000 seeds, clothianidin residues were <0.003-0.019 ppm in roots and <0.003-0.011 ppm in tops at normal crop maturity, 109-179 days after planting (DAP). Clothianidin residues were >LOQ in only 3 out of 24 root samples (0.015, 0.015, and 0.019 ppm), and 2 out of 24 samples of tops. Residues of TMG were non-detectable (ND, <0.001 ppm) in all root samples, and <0.003-0.026 ppm in samples of tops, with only 5 tops samples having TMG residues >LOQ. Average residues in roots and tops were, respectively, 0.011 and 0.010 ppm for clothianidin, and 0.010 and 0.011 ppm for TMG. The highest average field trial (HAFT) residues for clothianidin were 0.017 ppm in roots, and 0.011 ppm in tops.

The available sugar beet processing study is adequate, and indicates that clothianidin residues do not concentrate in refined sugar (<0.3X), but can concentrate in molasses (2.9X) and dried pulp (1.5X). Based on HAFT residues for clothianidin of 0.017 ppm in roots, and the above processing factors, the maximum expected residues would be 0.026 ppm in dried pulp, and 0.049 ppm in molasses.

Adequate livestock feeding studies are available for clothianidin, and tolerances for clothianidin residues in livestock commodities were recently assessed in conjunction with

petitions for uses on grapes, potatoes, sorghum, and cotton (D309473; William Drew; 2/1/2006). Based on the registered uses, the theoretical dietary burdens (TDB) of livestock for clothianidin residues are calculated to be 0.325 ppm for beef cattle, 0.264 ppm for dairy cattle, and 0.010 ppm for poultry and swine. For the current petition, the only livestock feedstuffs are sugar beet dried pulp and molasses; these feedstuffs have substantially lower residues than any of the feedstuffs they would replace in the calculated livestock diets. Therefore, the proposed use on sugar beets will not increase the TDB of livestock for clothianidin, and reassessment of livestock tolerances is not required for this petition.

Regarding the proposed use on sugar beets, regulatory requirements pertaining to clothianidin residues in rotational crops have been fulfilled, and the rotational crop restrictions on the proposed label are adequate. Bayer has submitted a rotational crop field trial study with clothianidin on soybeans in response to a data deficiency identified during HED's review of tolerance petition #1F6315 (D282446, Yan Donovan, 5/01/2003). The data deficiency was cited in section 860.1900 of Donovan's memorandum. HED has reviewed the submitted study, and concluded that it is acceptable for both scientific and regulatory purposes. The rotational soybean field trial data deficiency has thus been resolved.

Regarding international maximum residue limits (MRLs) for clothianidin, harmonization of the proposed tolerances in sugar beet roots, molasses, and dried pulp is not an issue, as there are no established or proposed Canadian, Mexican, or Codex MRLs for clothianidin residues in sugar beet commodities (See Appendix 1).

Regulatory Recommendations and Residue Chemistry Deficiencies

No major deficiencies were noted in the subject petition that would preclude the establishment of permanent tolerances for clothianidin residues in sugar beet roots, dried pulp, and molasses. Issues pertaining to the storage stability of TMG in representative potato commodities should be resolved (see below). The available data support permanent tolerances for clothianidin at 0.02 ppm in sugar beet roots, 0.03 ppm in sugar beet dried pulp, and 0.05 ppm in sugar beet molasses.

To support the stability of TMG residues in sugar beet commodities, the petitioner cited data from an ongoing study examining the stability of TMG in frozen sugar beet leaves, and potato tubers, flakes, and chips. These data should be submitted for evaluation.

The rotational soybean field trial data deficiency (identified during HED's review of Bayer's tolerance petition #1F6315) has been resolved.

Background

Clothianidin is a systemic insecticide belonging to both the chloronicotinyl (neonicotinoid) and nitroguanidine classes of chemicals. It is also a major metabolite of thiamethoxam. Clothianidin is currently registered to Bayer for use as a seed treatment on corn, canola (rapeseed), and sorghum, and to Arvesta Corporation for use as a foliar application on pome fruits, grapes, and potatoes. It enters the transpiration stream through the roots and cotyledons of newly germinating seedlings, and protects below- and above-ground plant parts from insect damage. Clothianidin binds (via ingestion and contact routes) with the nicotinic acetylcholine receptor sites, interfering with transmission of stimuli, and eventually inhibiting

reproduction of the insect.

Permanent tolerances have been established for residues of clothianidin in or on a variety of plant commodities at levels ranging from 0.01 ppm in several plant commodities to 1.0 ppm in pome fruits, and have also been established in milk at 0.01 ppm (40CFR §180.586[a]). Time-limited tolerances have been established at 0.02 ppm in sugar beet roots and tops (40CFR §180.586[b]), which expire on 12/31/2009. In addition, tolerances for indirect or inadvertent residues have been established for clothianidin at 0.02 ppm in non-grass livestock feeds; forage, fodder, and straw of cereal grains; grass forage, fodder, and hay; and soybean forage and hay (40CFR §180.586[d]). The ROC in plants, for purposes of both risk assessment and the tolerance expression, is parent clothianidin only. However, the Agency (D282446; Yan Donovan; 5/01/2003) has indicated that for new uses on root and/or leafy vegetable crops, samples should also be analyzed for residues of the metabolite, TMG. Alternatively, the registrant may submit additional metabolism data; preferably, side-by-side thiazol- and nitroimino- radiolabeled studies. Additionally, for rotational crop field trial studies, samples should be analyzed for parent, TZNG, and (if possible) MNG. In the absence of data for MNG, residues will be estimated using ratios from the confined study if levels are needed for dietary risk assessment.

Bayer has submitted a petition (PP#6F7159) proposing the use of Poncho Beta (with EPA File Symbol 264-RNLA) as a seed treatment on sugar beets. The proposed product is an MAI formulation containing 3.33 lb/gal of clothianidin, and 0.44 lb/gal of beta-cyfluthrin, formulated as an SE. To support this use, Bayer has submitted sugar beet field trials in which an SE formulation containing both clothianidin (4 lb ai/gal) and cyfluthrin (1 lb ai/gal) was applied as a seed treatment.

Bayer has also submitted a rotational crop field trial study with clothianidin on soybeans in response to a data deficiency identified during HED's review of tolerance petition #1F6315 (D282446, Yan Donovan, 5/01/2003).

The nomenclature of clothianidin and TMG are summarized in Table 1 (below), and the physicochemical properties of clothianidin are summarized in Table 2 (below).

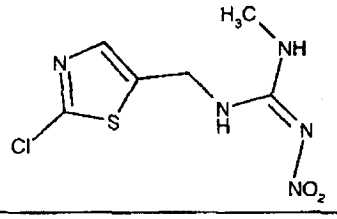
TABLE 1 Clothianidin Nomenclature.	
Compound	
Empirical Formula	$C_6H_8ClN_5O_2S$
Molecular Weight	249.7

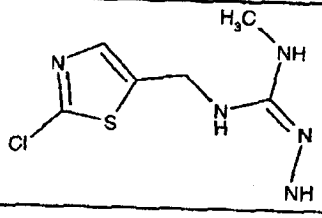
TABLE 1 Clothianidin Nomenclature.	
Common Name	Clothianidin
Company Experimental NameS	TM-444, TI-435, V-10066
IUPAC Name	*(E)*-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS Name	[*C(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine
CAS Registry Number	210880-92-5 (formerly 205510-53-8)
End-use Product (EP)	Poncho Beta (3.33 lb ai/gal SE; EPA File Symbol 264-RNLA)
Metabolite	
Common Name	TMG
IUPAC/CAS Name	*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methylguanidine
CAS registry Number	635283-91-9

TABLE 2 Physicochemical Properties of Clothianidin (TGAI*).			
Parameter	Value		Reference
Melting Point (°C)	176.8		MRID #45422301
pH at 23°C	6.24 (1% solution/suspension)		
Density (g/cm^3)	1.61 (PAI*), 1.59 (TGAI)		
Water Solubility (g/L) at 20°C	0.327		
Solvent Solubility (g/L) at 25°C	Acetone	15.2	
	Dichloromethane	1.32	
	Ethyl acetate	2.03	
	Heptane	<0.00104	
	Methanol	6.26	
	Octanol	0.938	
	Xylene	0.0128	
Vapor Pressure (Pa) at 20°C	1.3×10^{-10}		
Dissociation Constant (pK_a) at 20°C	11.09		
Octanol/Water Partition Coefficient, (Log K_{OW}) at 25°C	0.7		
UV/visible Absorption Spectrum (Maximum, nm)	265.5 (acidic or neutral) 246.0 (basic)		

* PAI = Purified Active Ingredient. TGAI = Technical Grade Active Ingredient.

860.1200 Directions for Use

Bayer is proposing the use of an MAI formulation containing 3.33 lb/gal of clothianidin, and 0.44 lb/gal of beta-cyfluthrin, formulated as an SE product. A copy of the proposed label was provided, and the proposed use on sugar beet seeds is summarized in Table 3, below.

TABLE 3 Summary of Directions for Use of Clothianidin.

Application Timing, Type, and Equipment [1]	Formulation [EPA File Symbol]	Use Rate (lb ai/100,000 Seeds)	Maximum # of Uses per Season	Maximum Seasonal Use Rate (lb ai/A) [2]	PHI (Days)	Use Directions and Limitations
Sugar beet						
Seed treatment.	3.33 lb ai/gal SE [3] [264-RNLA]	0.132	1	0.069-0.095	NS	All seeds treated must be conspicuously colored at the time of treatment. Do not use treated seed for food, feed, or oil processing.

1. To be used only in liquid or slurry seed-treating equipment by commercial seed treaters. Do not use in on-farm equipment designed for seed treatment at the time of planting.
2. Maximum field use rate, based on seeding rates of roughly 53,000-72,000 seeds per acre.
3. This formulation is an SE, which is a heterogeneous preparation consisting of a stable dispersion of the ai in the form of solid particles and fine globules in a continuous water phase. It is an MAI which also contains 0.44 lb/gal of beta-cyfluthrin.

Conclusion: The proposed label directions are adequate, and are supported by the available field trial data.

860.1300 Nature of the Residue – Plants

MARC Decision Memo D282449; Yan Donovan; 4/25/2003
Residue Chemistry Memo D282446; Yan Donovan; 5/1/2003

Adequate plant metabolism studies are available reflecting the application of [^{14}C]-clothianidin as a seed treatment to corn and sugar beets, as foliar applications to apples, and as soil and foliar applications to tomatoes. Based on these metabolism studies, HED concluded that the nature of the residue has been adequately delineated, and that parent only is the ROC to be used in risk assessment, and the tolerance expression, for primary crops. However, HED also determined that future new uses on root crops and/or leafy vegetables will require analysis for residues of TMG along with parent in field trial samples. The metabolic profiles in the tested primary crops were similar, in that the highest level residue was the parent, clothianidin, with the exception of mature sugar beet tops.

860.1300 Nature of the Residue - Livestock

MARC Decision Memo D282449; Yan Donovan; 4/25/2003
Residue Chemistry Memo D282446; Yan Donovan; 5/1/2003

The nature of clothianidin residues in livestock is understood, based on adequate goat and hen metabolism studies. In these studies, a goat was dosed orally for 3 days with [^{14}C]-clothianidin at levels equivalent to roughly 200 ppm in the diet, and hens were dosed orally for 3 days with [^{14}C]-clothianidin at levels equivalent to 140 ppm in the diet. For ruminants, HED concluded that the ROCs include parent, TZU, TZG, TZNG, and ATMG-Pyruvate, and that these residues should be included in risk assessments. For poultry, HED concluded that the ROCs include parent, TZU, TZG, TZNG and ATG-Acetate for risk assessment purposes. However, for

purposes of tolerance enforcement, HED recommended that only the parent compound needs to be included in the tolerance expression for livestock commodities.

860.1340 Residue Analytical Methods – Plants

PMV Results Memo D282448; Pat Schermerhorn; 7/17/2003

Adequate LC/MS/MS methods (Bayer Methods 00552, 00552-M001 and 109240-1) are available for enforcing tolerances of clothianidin residues in plant commodities. Bayer Method 00552-M001 is a modification of Method 00552; the modified method includes the use of an internal standard to quantitate clothianidin. The Analytical Chemistry Branch (ACB) of the Biological and Economic Analysis Division (BEAD) has approved these methods for tolerance enforcement (D282448; Pat Schermerhorn; 7/17/2003), and they were forwarded to FDA for inclusion in PAM Volume II. The validated method LOQ for clothianidin is either 0.010 or 0.020 ppm, depending on the matrix.

In the current field trials and processing studies, residues of clothianidin and its metabolite, TMG, were determined in sugar beet tops, roots, and processed fractions using another LC/MS/MS method (Bayer Method TI-002-P05-001). This method is similar to Bayer Method 00552-M001, with the principal difference being that Method TI-002-P05-001 also determines the metabolite, TMG.

For Method TI-002-P05-001, residues were extracted sequentially with ACN (twice), and ACN/water (twice; 1:1, v/v); the extracts were then combined and centrifuged. A mixed internal standard containing [$^2H_6$]-clothianidin and [$^2H_6$]-TMG was added, after which the residues were diluted with 0.045% acetic acid. Residues were then purified using a C_{18} SPE cartridge, concentrated, and re-dissolved in water/methanol (9:1, v/v, each containing 0.1% formic acid). Residues of clothianidin and TMG were then determined by LC/MS/MS using the following transitions for quantitation: clothianidin, m/z 250→169; [$^2H_6$]-clothianidin, m/z 253→172; TMG, m/z 207→134; and [$^2H_6$]-TMG, m/z 210→137. The presence of clothianidin and TMG residues was confirmed using the m/z 250→132 and m/z 207→71 transitions, respectively. Residues of clothianidin and TMG are expressed in clothianidin equivalents. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots, tops, and processed fractions, with the exception of clothianidin in molasses (0.02 ppm). The statistically calculated LODs for clothianidin are 0.003 ppm in roots, tops, refined sugar, and dried pulp, and 0.011 ppm in molasses. The statistically calculated LODs for TMG are 0.001 ppm in roots, 0.002 ppm in refined sugar and dried pulp, 0.003 ppm in tops, and 0.005 ppm in molasses.

This LC/MS/MS method was validated in conjunction with the analysis of the field trial and processing study samples using control samples fortified with each analyte at levels bracketing the observed residues.

Conclusion: The LC/MS/MS method (Bayer Method TI-002-P05-001) is adequate for collecting data on residues of clothianidin and TMG in sugar beet tops, roots, and processed commodities.

860.1340 Residue Analytical Methods – Livestock

PMV Results Memo D282448; Pat Schermerhorn; 7/17/2003

An adequate LC/MS/MS method (Bayer Method 00624) is available for determining residues of clothianidin, TZU, TZG, TZNG, and ATMG-pyruvate in milk. The method LOQ is 0.010 ppm for each analyte in milk. This method has been approved for tolerance enforcement by ACB (D282448; Pat Schermerhorn; 7/17/2003), and has been forwarded to FDA for inclusion in PAM Volume II.

860.1360 Multiresidue Methods

Multiresidue method testing of clothianidin and its metabolites, MNG, TZG, TZNG, TZU, and ATMG-Pyr have been submitted (D282446, Yan Donovan, 5/1/2003). Clothianidin and its major metabolites are not adequately recovered using any of the multiresidue methods. These data were forwarded to the US FDA for further evaluation.

860.1380 Storage Stability

Adequate storage stability data are available indicating that clothianidin is stable at -18°C for intervals of up to 24 months in sugar beet roots, corn (grain, forage, and straw), and canola seed (D282446; Yan Donovan; 5/1/2003), and for intervals of up to 15 months in apples and apple juice (D287182; William Cutchin; 7/13/2004). Data are also available indicating that residues of both clothianidin and TMG are stable at -20°C for intervals of up to 6 months in potatoes, and 5.3 months in grapes (D309473; William Drew; 2/1/2006).

In conjunction with the field trials and processing study, Bayer also provided summary data from an ongoing storage stability study in which control samples of sugar beet leaves, potato tubers, and potato flakes and chips were fortified with TMG at 0.2 ppm, and stored at less than -15°C. Duplicate stored samples were analyzed for TMG residues using Bayer Method TI-002-P05-001 after approximately 0, 1, 3, 6, and 12 months of frozen storage. Average corrected recoveries of TMG after 12 months of frozen storage were 94% from sugar beet leaves and potato tubers, 83% from potato flakes, and 95% from potato chips.

In the sugar beet field trials, samples of roots and leaves were stored at -15°C or colder for up to 380 days (12.5 months) prior to analysis. In the processing study, samples were stored at -15°C or colder for up to 24 days (refined sugar and dried pulp), 130 days (molasses), or 385 days (roots) prior to analysis.

Conclusion: **Provided that the storage stability study on TMG in sugar beet leaves and potatoes is submitted, and deemed adequate by the Agency, the available data will adequately support the storage durations and conditions for the current sugar beet field trials and processing study.**

860.1400 Water, Fish, and Irrigated Crops

This guideline requirement is not relevant to the current petition as the proposed use is non-aquatic.

860.1460 Food Handling

This guideline requirement is not relevant to the current petition as no uses are being proposed for food/feed handling establishments.

860.1480 Meat, Milk, Poultry, and Eggs

Adequate livestock feeding studies are available for clothianidin (D282446; Yan Donovan; 5/1/2003), and tolerances for clothianidin residues in livestock commodities were recently assessed in conjunction with petitions for uses on grapes, potatoes, sorghum, and cotton (D309473; William Drew; 2/1/2006). Under those petitions, the TDBs of livestock for clothianidin residues were calculated to be 0.321 ppm for beef cattle, 0.287 ppm for dairy cattle, 0.020 ppm for poultry, and 0.035 ppm for swine. Based on recent changes in Agency guidance on calculating residues in reasonably balanced livestock diets (memo of October 2006), the TDB for livestock to clothianidin residues was recalculated to be 0.325 ppm for beef cattle, 0.264 ppm for dairy cattle, and 0.010 ppm for swine and poultry (see Table 4, below).

The only livestock feedstuffs associated with the current petition are sugar beet dried pulp and molasses. As clothianidin residues in dried pulp and molasses are substantially lower than in the feedstuffs they would replace in the calculated diets, the proposed use on sugar beets will not increase the TDB of livestock for clothianidin residues. Therefore, reassessment of livestock tolerances is not required for this petition. The current 0.01 ppm tolerance in milk is adequate, and tolerances are not required in eggs nor the fat, meat, and meat byproducts of cattle, goats, hogs, horses, sheep, and poultry.

TABLE 4. Calculation of Theoretical Dietary Burdens of Clothianidin Residues to Livestock.

Feedstuff	Dietary Component	% Dry Matter[3]	% Diet[1]	Residues (ppm)[2]	Dietary Contribution (ppm)[3]
Beef Cattle					
Apple Pomace, Wet	CC	40	20	0.24[4]	0.120
Potato, Culls	CC	20	25	0.05	0.063
Canola, Meal	PC	88	15	0.01	0.002
Corn, Field, Forage	R	40	40	0.14[5]	0.140
TOTAL BURDEN			**100**		**0.325**
Dairy Cattle					
Apple Pomace, Wet	CC	40	10	0.24[4]	0.060
Potato, Processed Waste	CC	20	10	0.05	0.025
Corn, Field, Grain	CC	88	15	0.01	0.002
Canola, Meal	PC	88	15	0.01	0.002
Corn, Field, Forage	R	40	50	0.14[5]	0.175
TOTAL BURDEN			**100**		**0.264**

TABLE 4 Calculation of Theoretical Dietary Burdens of Clothianidin Residues to Livestock.					
Feedstuff	Dietary Component	% Dry Matter[1]	% Diet[1]	Residues (ppm)[2]	Dietary Contribution (ppm)[3]
Poultry and Swine					
Corn, Grain	CC	NA	80	0.01	0.008
Canola, Meal	PC	NA	20	0.01	0.002
TOTAL BURDEN			**100**		**0.010**

1. Potential dietary burdens of livestock were determined using recent Agency guidance on calculating reasonably balanced livestock diets (memo of October 2006).
2. The tolerance level for clothianidin in the associated raw agricultural commodity (RAC) was used unless otherwise indicated.
3. Contribution = ([Tolerance ÷ %Dry Matter] x %Diet) for beef and dairy cattle. Contribution = (Tolerance x %Diet) for poultry and swine.
4. Residues in wet apple pomace were estimated to be 0.24 ppm, based on the 1.0 ppm tolerance in pome fruit, and the 0.24X processing factor from the apple processing study.
5. As clothianidin is also a metabolite of thiamethoxam, the residues in corn forage include the proposed tolerance plus estimated residues of clothianidin resulting from the use of thiamethoxam.

860.1500 Crop Field Trials

DER for MRID #47007809 (Sugar Beet)

Bayer submitted field trials depicting the use of clothianidin (4 lb ai/gal SE) as a seed treatment on sugar beets. The results from these trials are discussed below, and summarized in Table 5, below.

TABLE 5 Summary of Residue Data from Sugar Beet Field Trials with Clothianidin.										
Crop Matrix	Use Rate[1] (lb ai/A)	DAP[2]	Analytes	Residue Levels (ppm)[3]						
				n	Min.	Max.	HAFT[4]	Median	Mean	Std. Dev.
Roots	0.070-0.094	109-179	Clothianidin	24	<0.010	0.019	0.017	0.010	0.011	0.002
			TMG		<0.010	<0.010	0.010	0.010	0.010	0
			Combined		<0.020	<0.029	0.027	0.020	0.021	0.002
Tops	0.070-0.094	109-179	Clothianidin	24	<0.010	0.011	0.011	0.010	0.010	0
			TMG		<0.010	0.026	0.024	0.010	0.011	0.004
			Combined		<0.020	<0.036	0.034	0.020	0.021	0.004

1. Clothianidin use rate was 0.132 lb ai/100,000 seeds; field use rate was calculated based on actual seeding rates.
2. DAP = Days After Planting; no PHI is proposed, as the application is a seed treatment.
3. Expressed in clothianidin equivalents. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and tops. For calculating the median, mean and standard deviation, the LOQ was used for residue values of <LOQ.
4. HAFT = Highest Average Field Trial.

Twelve sugar beet field trials were conducted in Zones 5, 7, 8, 9, 10, and 11 during 2004. For each trial, seeds were treated using a commercial Hege 11 Seed Treater, with an SE formulation containing both clothianidin (4 lb ai/gal) and cyfluthrin (1 lb ai/gal). Seeds were treated at a rate equivalent to 0.132 lb of clothianidin per 100,000 seeds. Based on the actual seeding rates used in the field trials (52,870-71,320 seeds/A), this rate was equivalent to 0.070-0.094 lb ai/A for clothianidin. Single control, and duplicate treated samples of sugar beet roots and tops were harvested from each trial at the earliest possible commercial maturity, 109-179

DAP. Samples were stored frozen for up to 380 days prior to analysis, a duration supported by available storage stability data.

The LC/MS/MS method (Bayer Method TI-002-P05-001) used to determine residues of clothianidin and its metabolite, TMG, in sugar beet roots and tops was adequately validated in conjunction with the analysis of field trial samples. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and tops, while the statistically calculated LODs are 0.003 ppm for clothianidin in both roots and tops, and 0.001 and 0.003 ppm for TMG in roots and tops, respectively.

Following a seed treatment with clothianidin (SE) at 0.132 lb ai/100,000 seeds, clothianidin residues were ND-0.019 ppm in roots, and ND-0.011 ppm in tops, at normal crop maturity. Clothianidin residues were >LOQ in only 3 out of 24 root samples, and 2 out of 24 tops samples. Residues of TMG were ND (<0.001 ppm) in all root samples, and ND-0.026 ppm in samples of tops, with only 5 tops samples having residues >LOQ. Combined residues were <0.020-<0.029 ppm in roots, and <0.020-<0.036 ppm in tops. Average residues in roots and tops, respectively, were 0.011 and 0.010 ppm for clothianidin, and 0.010 and 0.011 ppm for TMG. Average combined residues were 0.021 ppm for both roots and tops. As the application was a seed treatment, no residue decline study was conducted.

Conclusions: The sugar beet field trial data are adequate, and support the proposed use pattern. Adequate numbers of trials were conducted in the appropriate geographical regions, and samples were analyzed for both ROCs using an adequate method. Provided that the storage stability data for TMG in sugar beet leaves and potatoes are submitted, and deemed adequate, the sample storage conditions and durations are also supported by the available storage stability data. The clothianidin residue data support the proposed tolerance of 0.02 ppm in sugar beet roots. Although residue data were also submitted for sugar beet tops, the Agency no longer considers sugar beet tops to be a significant livestock feedstuff; therefore, a separate tolerance in tops is not required.

860.1520 Processed Food and Feed

DER for MRID #47007810 (Sugar Beet)

Bayer submitted a sugar beet processing study supporting the proposed seed treatment use. The results from this study are discussed below, and summarized in Table 6, below.

TABLE 6 Summary of Processing Factors for Clothianidin

RAC[1]	Processed Commodity	Processing Factor		
		Clothianidin	TMG	Combined
Sugar Beet Roots	Refined Sugar	<0.3X	NA[2]	<0.3X
	Dried Pulp	1.5X	NA	1.3X
	Molasses	2.9X	NA	2.0X

1. RAC = Raw Agricultural Commodity.
2. NA = Not Applicable (residues of TMG were ND in all commodities).

In this study, sugar beet seeds were treated with an SE formulation containing both clothianidin (4 lb ai/gal) and cyfluthrin (1 lb ai/gal). The treated seeds were planted at a field

trial site in MN during 2004. The application rate for clothianidin was equivalent to 0.661 lb ai/100,000 seeds (5X rate), or 0.350 lb ai/A, based on the actual seeding rate used at the trial site (52,900 seeds/A). Single bulk control and treated samples of sugar beet roots were harvested at normal commercial maturity, 147 DAP, and placed in frozen storage. After roughly 13 months of storage, roots were processed into molasses, refined sugar, and dried pulp using simulated commercial procedures. Root samples were stored frozen for 12.6 months prior to analysis, and the frozen processed commodities were analyzed within 1 month (sugar and dried pulp) or 4.3 months (molasses) of collection. These storage durations are supported by the available storage stability data.

The LC/MS/MS method (Bayer Method TI-002-P05-001) used to determine residues of clothianidin and its metabolite, TMG, in sugar beet roots and processed commodities was adequately validated in conjunction with the analysis of processing study samples. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and processed commodities, with the exception of clothianidin in molasses (0.02 ppm). The statistically calculated LODs for clothianidin are 0.003 ppm in roots, sugar, and dried pulp, and 0.011 ppm in molasses. The statistically calculated LODs for TMG are 0.001 ppm in roots, 0.002 ppm in sugar and dried pulp, and 0.005 ppm in molasses.

Following a seed treatment at a 5X rate, residues of clothianidin averaged 0.011 ppm in roots (the RAC) at maturity, and averaged <0.003 ppm (<LOD) in refined sugar, 0.017 ppm in dried pulp, and 0.032 ppm in molasses. Residues of TMG were non-detectable in roots and all processed fractions. Processing factors for clothianidin were <0.3X in refined sugar, 1.5X in dried pulp, and 2.9X in molasses. Processing factors for combined residues were similar, but slightly lower, as TMG residues were <LOD in all fractions.

Conclusions: Provided that the supporting storage stability data are submitted, the sugar beet processing study is adequate. Based on the clothianidin residue data, residues do not concentrate in refined sugar (<0.3X), but concentrate by 1.5X in dried pulp, and 2.9X in molasses. The maximum possible processing factor for sugar beet dried pulp is 20X.

860.1650 Submittal of Analytical Reference Standards

An analytical reference standard for clothianidin is available (as of 4/9/2007) at the EPA National Pesticide Standards Repository.

860.1850 Confined Accumulation in Rotational Crops

An adequate confined rotational crop study is available, and was reviewed in conjunction with the petition for use on canola and corn (D282446; Yan Donovan; 5/1/2003). Rotational crops of turnips, Swiss chard, and wheat were planted approximately 1, 5, and 10 months following a single soil application of [^{14}C]-clothianidin at the rate of 0.293 lb ai/A (3.1X sugar beet rate), and samples of the appropriate RACs were harvested at maturity. The metabolism of clothianidin in rotational crops was similar to that in the primary crops, and HED concluded that parent, TZNG, and MNG are the ROCs in rotational crops for risk assessment, but that only the parent compound needs to be included in the tolerance expression.

860.1900 Field Accumulation in Rotational Crops

Adequate limited field rotational crop studies are available, and were reviewed in conjunction with the petition for seed treatment uses on canola and corn (D282446; Yan Donovan; 5/1/2003). In these studies, 10-11 trials were conducted in which mustard greens, turnips, and wheat were planted 1, 4, 8, and 12 months following a single seed treatment to corn at rates equivalent to 0.144-0.171 lb ai/A (1.5-1.8X the proposed rate for sugar beets).

Based on the residue data from these field trials, tolerances were established for clothianidin residues at 0.02 ppm in non-grass livestock feeds (group 18); the forage, fodder, and straw of cereal grains (group 16); grass forage, fodder, and hay (group 17); and soybean forage and hay. The resulting label for corn and canola uses specified a rotational crop restriction of 30 days for the above crops/crop groups, and a 1-year restriction for other crops not on the label.

The proposed use directions for sugar beets allow for immediate replanting with cotton, corn, sorghum, rapeseed, and canola, all of which have registered clothianidin uses. The proposed label specifies a rotational crop restriction of 30 days for cereal grains, soybeans, dried beans and peas, and 1 year for all other crops. These restrictions are supported by the available field rotational crop trials.

Additionally, Bayer has submitted a rotational crop field trial study with clothianidin on soybeans in response to a data deficiency identified during HED's review of tolerance petition #1F6315 (D282446, Yan Donovan, 5/01/2003). The data deficiency was cited in section 860.1900 of Donovan's memorandum as follows:

> The petitioner has submitted a revised Section F to establish tolerances on cereal grain, forage, fodder and straw (Group16), grass forage, fodder and hay (Group 17), animal feed, nongrass (Group 18), and soybean forage and hay, all at 0.02 ppm. HED concludes that based on rotational crop field data, the requested 30 day plantback restrictions are adequate. The 30 day PBI's for soybeans and dried beans are conditional upon the submission of two rotational crop field trials showing residues of clothianidin and the TZNG metabolite in mature soybeans are each <0.01 ppm.

HED has reviewed the submitted study, and concluded that it is acceptable for both scientific and regulatory purposes. **The rotational soybean field trial data deficiency (identified during HED's review of Bayer's tolerance petition #1F6315) has thus been resolved.**

860.1550 Proposed Tolerances

For purposes of setting tolerances, the Agency has determined that clothianidin is the ROC in both primary and rotational crops, as well as in livestock commodities. Permanent tolerances are established for residues of clothianidin in a variety of plant commodities at levels ranging from 0.01 ppm to 1.0 ppm, and in milk at 0.01 ppm (40CFR §180.586[a]). Time-limited tolerances have been established at 0.02 ppm in sugar beet roots and tops (40CFR §180.586[b]), which expire on 12/31/2009. In addition, tolerances for indirect or inadvertent residues have been established for clothianidin at 0.02 ppm in/on non-grass livestock feeds, the forage, fodder and straw of cereal grains, grass forage, fodder and hay, and soybean forage and hay (40CFR

§180.586[d]). The proposed and recommended tolerances in sugar beet commodities are listed in Table 7, below.

In the sugar beet field trials, clothianidin residues were ND-0.019 ppm in roots, with only 3 out of 24 samples having residues above the LOQ (0.015, 0.015, and 0.019 ppm), and residues of TMG were ND (<0.001 ppm) in all root samples. Because 21 of the 24 sugar beet root samples had residues that were <LOQ, use of the MRL/tolerance calculator was not appropriate. Utilizing the CA Method ($\mu + 3\sigma$) would result in a recommended tolerance of 0.017 ppm. However, based on the maximum clothianidin residues observed in roots (0.019 ppm), the proposed tolerance of 0.02 ppm in sugar beet roots is adequate. No tolerance is required in sugar beet tops, as sugar beet tops are no longer a regulated livestock feedstuff.

The results from the sugar beet processing study indicate that a separate tolerance is not required in refined sugar, as clothianidin residues did not concentrate in sugar (<0.3X). However, clothianidin residues concentrated by 1.5X in dried pulp, and by 2.9X in molasses. Based on HAFT residues of 0.017 ppm for clothianidin in sugar beet roots, and the above processing factors, the maximum expected residues would be 0.026 ppm in dried pulp, and 0.049 ppm in molasses. These data would support tolerances of 0.03 ppm in dried pulp, and 0.05 ppm in molasses.

Tolerances for clothianidin residues in livestock commodities were recently reassessed in conjunction with petitions for uses on grapes, potatoes, sorghum, and cotton (D309473; William Drew; 2/1/2006). As the proposed sugar beet tolerances do not alter the TDB of livestock for clothianidin residues, reassessment of livestock tolerances is not required for this petition, and the current tolerance in milk is adequate.

Regarding international MRLs for clothianidin, harmonization of the proposed tolerances for sugar beet roots, molasses, and dried pulp is not an issue, as there are no established or proposed Canadian, Mexican, or Codex MRLs for clothianidin residues in sugar beet commodities (See Appendix 1).

TABLE 7 Tolerance Summary for Clothianidin.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments [*Correct Commodity Definition*]
Beet, sugar, roots	0.02	0.02	Adequate data are available.
Beet, sugar, tops	0.04	None	Not a regulated food/feed item.
Beet, sugar, molasses	0.06	0.05	Based on HAFT residues of 0.017 ppm in roots, along with processing factors of 1.5X for dried pulp, and 2.9X for molasses, the maximum expected residues would be 0.026 ppm in dried pulp, and 0.049 ppm in molasses.
Beet, sugar, dried pulp	None	0.03	

REFERENCES

PP#1F06315. TI-435 (Clothianidin) on Corn and Canola. Summary of Analytical Chemistry and Residue Data.; D282446; Yan Donovan; 5/01/2003.

Method Review - PP#1F06315. New Chemical - Clothianidin (TI-435) in/on Corn and Canola. Original Request for Petition Method Validation.; D282448; Pat Schermerhorn; 7/17/2003.

Clothianidin. Petition for the Permanent Tolerances for the Uses on Apples and Pears and for the Use on Tobacco. Summary of Analytical Chemistry and Residue Data.; D287182; William Cutchin; 7/13/2004.

Clothianidin. Tolerance Petition Requesting Food Use of the Insecticide Clothianidin on Grapes, Potatoes, Sorghum, and Cotton (Section 3 Registration). Summary of Analytical Chemistry and Residue Data.; D303164, D309473, D309474, D312449, D314533, D318496; William Drew; 2/1/2006.

ATTACHMENTS

APPENDIX 1 - International Residue Limit Status sheet

APPENDIX 1. International Tolerances.

INTERNATIONAL RESIDUE LIMIT STATUS			
Chemical Name: *(E)-N-*[(2-Chloro-5-thiazolyl) methyl]-*N'*-methyl-*N''*-nitroguanidine	**Common Name:** Clothianidin	**X Recommended tolerances** ☐ Reevaluated tolerance ☐ Other	**Date:** 5/16/2007

Codex Status (Maximum Residue Limits)		**US Tolerances**	
X No Codex proposal step 6 or above ☐ No Codex proposal step 6 or above for the crops requested		**Petition Number: 6F7159** **DP Number: 335353** **Other Identifier: PC Code 044309**	
Residue definition (step 8/CXL): NA		**Reviewer/Branch: William T. Drew/RAB2**	
		Residue definition: Clothianidin	
Crop(s)	**MRL (mg/kg)**	**Crops**	**Tolerance (ppm)**
		Beet, sugar, roots	0.02
		Beet, sugar, dried pulp	0.03
		Beet, sugar, molasses	0.05

Limits for Canada		**Limits for Mexico**	
X No Limits ☐ No Limits for the crops requested		X No Limits ☐ No Limits for the crops requested	
Residue definition: Clothianidin		Residue definition: NA	
Crop(s)	**MRL (mg/kg)**	**Crop(s)**	**MRL (mg/kg)**

NOTES: per Steve Funk (5/16/2007). NA = Not Applicable.

Primary Evaluator: William T. Drew, Chemist, HED/RAB2 — Date: 5/2/2007

Peer Reviewer: Douglas Dotson (PhD), Chemist, HED/RAB2 — Date: 5/17/2007

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Road, Building 100, Suite B, Durham NC 27713). It has been reviewed by the Health Effects Division (HED), and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT

MRID #47007809. Francis K. Duah, Ann M. Harbin (2006) *Clothianidin + Cyfluthrin 600 SE – Magnitude of the Residue in/on Sugar Beet.* Laboratory Project #1625. Bayer Study #RATIY006. Unpublished study prepared by Bayer CropScience. 238 pages. {OPPTS Residue Chemistry Test Guideline 860.1500}

EXECUTIVE SUMMARY

Bayer CropScience submitted field trials supporting the use of clothianidin as a seed treatment for sugar beets. Twelve sugar beet field trials were conducted in Zones 5, 7, 8, 9, 10, and 11 during 2004. For each trial, seeds were treated (using a commercial Hege 11 Seed Treater) with a suspo-emulsion (SE) formulation containing both clothianidin, at 4 pounds of active ingredient per gallon (lb ai/gal), and cyfluthrin (1 lb ai/gal). Cyfluthrin is not addressed in this document. Seeds were treated at a rate equivalent to 0.132 lb clothianidin per 100,000 seeds. Based on the actual seeding rates used in the field trials, 52,870-71,320 seeds per acre (seeds/A), this rate was equivalent to 0.070-0.094 lb ai/A for clothianidin. Single control, and duplicate treated samples of sugar beet roots and tops were harvested from each trial at the earliest possible commercial maturity, 109-179 days after planting (DAP). Samples were stored frozen for up to 380 days prior to analysis, a duration supported by available storage stability data.

The LC/MS/MS method (Bayer Method TI-002-P05-001) used to determine residues of clothianidin and its metabolite, TMG, in sugar beet roots and tops was adequately validated in conjunction with the analysis of field trial samples. For this method, residues were extracted sequentially with acetonitrile (ACN), and ACN/water, then fortified with internal standards of [$^2H_6$]-clothianidin and [$^2H_6$]-TMG. The combined extract was acidified, and residues were purified by C_{18} solid phase extraction (SPE) prior to LC/MS/MS analysis. Residues of clothianidin and TMG are expressed in clothianidin equivalents. The validated limit of quantitation (LOQ) for both clothianidin and TMG is 0.010 ppm in sugar beet roots and tops, and the statistically calculated limits of detection (LODs) are 0.003 ppm for clothianidin in both roots and tops, and 0.001 and 0.003 ppm for TMG in roots and tops, respectively.

Following a seed treatment with clothianidin (SE) at 0.132 lb ai per 100,000 seeds, clothianidin residues were non-detectable (ND, <0.003 ppm) to 0.019 ppm in roots, and ND (<0.003 ppm) to 0.011 ppm in tops at normal crop maturity. Clothianidin residues were >LOQ (>0.010 ppm) in only 3 out of 24 root samples, and 2 out of 24 tops samples. Residues of TMG were ND (<0.001 ppm) in all root samples, and ND (<0.003 ppm) to 0.026 ppm in samples of tops, with only 5 tops samples having residues >LOQ (>0.010 ppm). Combined residues of clothianidin and TMG were <0.020-<0.029 ppm in roots, and <0.020-<0.036 ppm in tops. Average residues in roots and tops were, respectively, 0.011 and 0.010 ppm for clothianidin, and 0.010 and 0.011 ppm for TMG. Average combined residues were 0.021 ppm for both roots and tops.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters used in the study, the sugar beet field trial residue data are classified as scientifically acceptable, provided that the 12-month storage stability data for TMG in sugar beet leaves and potato tubers are submitted and deemed adequate. The acceptability of this study for regulatory purposes is addressed in the US EPA Residue Chemistry Summary Document (DP #335355).

COMPLIANCE

Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an adverse impact on the validity of the study.

A. BACKGROUND INFORMATION

Clothianidin is a systemic insecticide belonging to both the chloronicotinyl (neonicotinoid) and nitroguanidine classes of chemicals. It is also a major metabolite of thiamethoxam. Clothianidin is currently registered to Bayer for use as a seed treatment on corn, canola (rapeseed), and sorghum, and to Arvesta Corporation for use as a foliar application on pome fruits, grapes, and potatoes. It enters the transpiration stream through the roots and cotyledons of newly germinating seedlings, and protects below- and above-ground plant parts from insect damage. Clothianidin binds (via ingestion and contact routes) with the nicotinic acetylcholine receptor sites, interfering with transmission of stimuli, and eventually inhibiting reproduction of the insect. Tolerances have been established for residues of clothianidin in or on plant commodities and milk at levels of 0.01-1.0 ppm (40CFR §180.586), including time-limited tolerances of 0.02 ppm in sugar beet root and tops, which expire on 12/31/2009. The residue of concern, for purposes of both risk assessment and the tolerance expression, is parent clothianidin only. However, the Agency (D282446, Yan Donovan, 5/01/2003) has indicated that for new uses on root and/or leafy vegetable crops, samples should also be analyzed for residues of the metabolite, TMG.

Bayer has submitted a petition (PP#6F7159) proposing the use of Poncho Beta (with EPA File Symbol 264-RNLA) as a seed treatment on sugar beets. The proposed product is a multiple active ingredient (MAI) formulation containing 3.33 lb/gal of clothianidin, and 0.44 lb/gal of beta-cyfluthrin, formulated as an SE. To support this use, Bayer has submitted sugar beet field trials in which an SE formulation containing both clothianidin (4 lb ai/gal) and cyfluthrin (1 lb ai/gal) was applied as a seed treatment.

The nomenclature of clothianidin and TMG are summarized in Table A.1 (below), and the physicochemical properties of clothianidin are summarized in Table A.2 (below).

TABLE A.1 Clothianidin Nomenclature.

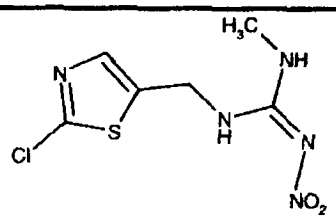
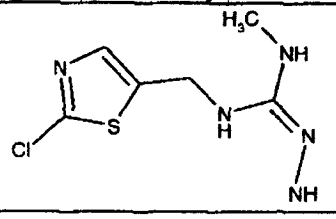
Compound	
Empirical Formula	$C_6H_8ClN_5O_2S$
Molecular Weight	249.7
Common Name	Clothianidin
Company Experimental Names	TM-444, TI-435, V-10066
IUPAC Name	*(E)*-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS Name	[*C(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine
CAS Registry Number	210880-92-5 (formerly 205510-53-8)
End-use Product (EP)	Poncho Beta (3.33 lb ai/gal SE; EPA File Symbol 264-RNLA)
Metabolite	
Common Name	TMG
IUPAC/CAS Name	*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methylguanidine
CAS registry Number	635283-91-9

TABLE A.2 Physicochemical Properties of Clothianidin (TGAI*).

Parameter	Value	Reference
Melting Point (°C)	176.8	MRID #45422301
pH at 23°C	6.24 (1% solution/suspension)	
Density (g/cm^3)	1.61 (PAI*), 1.59 (TGAI)	
Water Solubility (g/L) at 20°C	0.327	

TABLE A.2 Physicochemical Properties of Clothianidin (TGAI*).

Parameter	Value		Reference
Solvent Solubility (g/L) at 25°C	Acetone	15.2	
	Dichloromethane	1.32	
	Ethyl acetate	2.03	
	Heptane	<0.00104	
	Methanol	6.26	
	Octanol	0.938	
	Xylene	0.0128	
Vapor Pressure (Pa) at 20°C	1.3×10^{-10}		
Dissociation Constant (pK_a) at 20°C	11.09		
Octanol/Water Partition Coefficient, (Log K_{OW}) at 25°C	0.7		
UV/visible Absorption Spectrum (Maximum, nm)	265.5 (acidic or neutral) 246.0 (basic)		

* PAI = Purified Active Ingredient. TGAI = Technical Grade Active Ingredient.

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

TABLE B.1.1 Trial Site Conditions.

Location (City, State; Year)	Soil Characteristics			
	Type	%OM	pH	CEC (meq/g)
Springfield, NE; 2004	Silt loam	2.9	6.2	14.1
Sabin, MN; 2004	Slit	3.5	7.9	25
Theilman, MN; 2004	Sandy loam	3.4	6.8	23
Northwood, ND; 2004	Loam	3.2	7.2	23.8
Campbell, MN; 2004	Clay loam	5.5	7.4	33.3
Velva, ND; 2004	Loam	4.1	6.2	21.9
Larned, KS; 2004	Sandy loam	1.5	7.2	9.2
Eaton, CO; 2004	Sandy clay loam	1.4	8.2	28.6
Fresno, CA; 2004	Sandy loam	0.52	7.3	3.8
Live Oak, CA; 2004	Clay loam	1.6	5.3	20.9
Madras, OR; 2004	Loam	1.3	7.1	23
Ephrata, WA; 2004	Sandy Loam	1.7	7.8	12.8

Average monthly minimum and maximum temperatures, and monthly precipitation and irrigation totals were reported for each site during the trial period. Temperatures and rainfall were compared to 10-year historical data. With the exception of three trial sites, no unusual weather conditions were noted. Unusual temperature extremes were noted at one site (#004) and higher than normal rainfall was noted at two sites (#005 and #007). However, residue levels in crop samples from these sites were similar to residue levels from the other trials; therefore, the

study authors concluded that the unusual weather conditions did not adversely affect the field trial results. The trials were conducted according to normal agricultural practices for the different regions, and information was provided on maintenance pesticides and fertilizers used at each site.

Two varieties of sugar beet seeds were treated at the Bayer CropScience Seed and Technology Center (McKinney, TX) using a commercial Hege 11 Seed Treater. The SE formulation containing 4 lb/gal of clothianidin, and 1 lb/gal of cyfluthrin was mixed with a seed colorant, and diluted with water to form a slurry for application. After drying, the treated seeds were shipped to the various field trial sites for planting.

TABLE B.1.2 Study Use Pattern.

Location (City, State; Year) [Trial ID]	End-use Product[1]	Application Information			
		Method[2]; Timing	Spray Volume	Rate[3] (lb ai/ 100,000 seeds)	Field Rate[3] (lb ai/A)[4]
Springfield, NE; 2004 [001]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.094
Sabin, MN; 2004 [002]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.070
Theilman, MN; 2004 [003]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.084
Northwood, ND; 2004 [004]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.079
Campbell, MN; 2004 [005]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.093
Velva, ND; 2004 [006]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.078
Larned, KS; 2004 [007]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.090
Eaton, CO; 2004 [008]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.088
Fresno, CA; 2004 [009]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.079
Live Oak, CA; 2004 [010]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.078
Madras, OR; 2004 [011]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.079
Ephrata, WA; 2004 [012]	4 lb ai/gal SE	Seed treatment.	N/A	0.132	0.088

1. The SE formulation is an MAI containing 4 lb/gal of clothianidin, and 1 lb/gal of cyfluthrin.
2. Seeds were treated using a commercial Hege 11 Seed Treater.
3. Rates are for clothianidin.
4. Field application rates were calculated using the actual seeding rate at each trial site (52,780-71,320 seeds/A).

TABLE B.1.3 Trial Numbers and Geographical Locations.

NAFTA Growing Zones	Sugar Beets		
	Submitted	Requested	
		Canada	US
1	--	--	--
2	--	--	--
3	--	--	--
4	--	--	--
5	5	2	5
6	--	--	--
7	1	--	1
7A	--	2	--
8	1	--	1

TABLE B.1.3 Trial Numbers and Geographical Locations.			
NAFTA Growing Zones	Sugar Beets		
	Submitted	Requested	
		Canada	US
9	1	--	1
10	2	--	2
11	2	--	2
12	--	--	--
13	--	--	--
14	--	1	--
Total	12	5	12

B.2. Sample Handling and Preparation

Single control, and duplicate treated samples (at least 1 kg per sample) of sugar beet roots and tops were harvested at the earliest possible date for commercial harvest (BBCH 49), 109-179 DAP. Samples were frozen within 1 hour of collection, and stored frozen until shipment by freezer truck to Bayer Research Park (in Stilwell, KS), where samples were stored at -15°C or colder. Samples were later shipped by freezer truck to the analytical laboratory, Pyxant Labs (in Colorado Springs, CO), where samples were homogenized with dry ice, and stored at -20°C or colder until analysis.

B.3. Analytical Methodology

Residues of clothianidin and TMG in sugar beet tops and roots were determined using an LC/MS/MS method (Bayer Method TI-002-P05-001). This method is similar to another LC/MS/MS method (Bayer Method 00552-M001) which has been approved for enforcing tolerances on plant commodities (D282448, Pat Schermerhorn, 7/17/2003). The principal difference between these methods is that Method TI-002-P05-001 also determines the metabolite, TMG.

For this method, residues were extracted sequentially with ACN twice, and ACN/water (1:1, v/v) twice; the extracts were then combined and centrifuged. A mixed internal standard containing [$^2H_6$]-clothianidin and [$^2H_6$]-TMG was added, and residues were diluted with 0.045% acetic acid. Residues were then purified using a C_{18} SPE cartridge, concentrated, and re-dissolved in water/methanol (9:1, v/v, each containing 0.1% formic acid). Residues of clothianidin and TMG were then determined by LC/MS/MS using the following transitions for quantitation: clothianidin, m/z 250→169; [$^2H_6$]-clothianidin, m/z 253→172; TMG, m/z 207→134; and [$^2H_6$]-TMG, m/z 210→137. The presence of clothianidin and TMG residues was confirmed using the m/z 250→132 and m/z 207→71 transitions, respectively. Residues of clothianidin and TMG are expressed in clothianidin equivalents. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and tops, while the statistically calculated LODs are 0.003 ppm for clothianidin in both roots and tops, and 0.001 and 0.003 ppm for TMG in roots and tops, respectively.

The above method was validated in conjunction with the analysis of the field trial samples using control samples of roots and tops fortified with clothianidin at 0.010 and 0.030 ppm, and TMG at 0.010 and 0.020 ppm.

C. RESULTS AND DISCUSSION

Twelve sugar beet field trials were conducted in Zones 5, 7, 8, 9, 10, and 11 during 2004. For each trial, sugar beet seeds were treated using a commercial seed treater (Hege 11) with an MAI formulation, containing both clothianidin (4 lb ai/gal SE) and cyfluthrin (1 lb ai/gal SE), at a rate equivalent to 0.132 lb ai (clothianidin) per 100,000 seeds. Based on the actual seeding rates used in the field trials, this rate was equivalent to 0.070-0.094 lb ai /A. Single control, and duplicate treated samples of sugar beet roots and tops were harvested from each trial site at the earliest possible commercial maturity, 109-179 DAP.

Samples were stored at -15°C or colder for up to 380 days prior to analysis (see Table C.2, below). Storage stability data are available indicating that clothianidin is stable at -18°C for intervals of up to 24 months in sugar beet roots, and corn forage (D282446, Yan Donovan, 5/3/2003). The study report also presented summary data from an on-going storage stability study examining the stability of TMG in potato commodities (tubers, flakes, chips), and sugar beet leaves at -15°C or colder. Although this study has not yet been reviewed by the Agency, the summary data indicate that TMG is stable in frozen potato tubers and sugar beet leaves for intervals of up to 373 days (12 months). Provided that the TMG study is deemed adequate by the Agency, these data will adequately support the storage durations and conditions for the current sugar beet field trials.

The LC/MS/MS method (Bayer Method TI-002-P05-001) used for determining residues of clothianidin and TMG was adequately validated in conjunction with the analysis of field trial samples. The average recovery (± standard deviation) of clothianidin was 98 ± 10% from tops, and 96 ± 9% from roots (see Table C.1, below). The average recovery (± standard deviation) for TMG was 88 ± 13% from tops, and 96 ± 10% from roots. The validated LOQ for both clothianidin and TMG, expressed in clothianidin equivalents, is 0.010 ppm in sugar beet roots and tops. The statistically calculated LODs are 0.003 ppm for clothianidin in both roots and tops, and 0.001 and 0.003 ppm for TMG in roots and tops, respectively. Apparent residues of both analytes were non-detectable in all control samples. Adequate sample calculations and example chromatograms were provided.

Following a seed treatment with clothianidin (SE) at 0.132 lb ai/100,000 seeds, clothianidin residues were ND (<0.003 ppm) to 0.019 ppm in roots, and ND (<0.003 ppm) to 0.011 ppm in tops at normal crop maturity (see Table C.3, below). Clothianidin residues were >LOQ (>0.010 ppm) in only 3 out of 24 root samples, and 2 out of 24 tops samples. Residues of TMG were ND (<0.001 ppm) in all root samples, and ND (<0.003 ppm) to 0.026 ppm in tops samples, with only 5 tops samples having residues >LOQ (>0.010 ppm). Combined residues were <0.020-<0.029 ppm in roots, and <0.020-<0.036 ppm in tops. Average residues in roots and tops were 0.011 and 0.010 ppm for clothianidin, and 0.010 and 0.011 ppm for TMG (see

Table C.4, below), while average combined residues were 0.021 ppm for both roots and tops. Because the proposed use is as a seed treatment, no residue decline data are required.

Common cultural practices were used to maintain plants; the weather conditions, maintenance chemicals, and fertilizer used in the study did not have a notable impact on the residue data.

TABLE C.1 Summary of Concurrent Recoveries of Clothianidin and TMG from Sugar Beet Roots and Tops.

Crop [Matrix]	Spike Level (ppm)	Sample Size (n)	Recoveries (%)	Mean ± Std. Dev. (%)
Clothianidin				
Sugar Beet [Tops]	0.010	10	84, 103, 103, 96, 83, 104, 87, 93, 106, 111	97 ± 10
	0.030	3	101, 113, 94	103 ± 10
	Total	**13**	**83-111**	**98 ± 10**
Sugar Beet [Root]	0.010	10	89, 88, 90, 104, 109, 96, 89, 90, 102, 80	94 ± 9
	0.030	3	102, 109, 96	102 ± 7
	Total	**13**	**80-109**	**96 ± 9**
TMG				
Sugar Beet [Tops]	0.010	10	91, 100, 115, 96, 84, 89, 102, 89, 92, 78	94 ± 10
	0.020	3	70, 72, 72	71 ± 1
	Total	**13**	**70-115**	**88 ± 13**
Sugar Beet [Root]	0.010	10	88, 88, 101, 94, 93, 95, 96, 93, 95, 92	94 ± 4
	0.020	3	80, 112, 119	104 ± 21
	Total	**13**	**80-119**	**96 ± 10**

TABLE C.2 Summary of Storage Conditions.

Residue	Crop [Matrix]	Storage Temperature (°C)	Maximum Storage Duration (Days)*	Interval of Demonstrated Storage Stability (Days)
Clothianidin	Sugar Beet [Root]	-15	380	730
	Sugar Beet [Tops]			730
TMG	Sugar Beet [Root]			373
	Sugar Beet [Tops]			373

* Duration from harvest to analysis. Extracts were stored for up to 3 day prior to analysis.

TABLE C.3 Residue Data from Sugar Beet Field Trials with Clothianidin.

Location (City, State; Year) [Trial ID]	Zone	Variety	Matrix	Total Use Rate (lb ai/A)	Harvest (DAP)[1]	Residues (ppm)[2]		
						Clothianidin	TMG	Combined[3]
Springfield, NE 2004 [001]	5	Beta 101	Root	0.094	143	[0.004][4], [0.004]	ND, ND	<0.020, <0.020
			Tops			[0.006], [0.004]	[0.008], [0.005]	<0.020, <0.020

TABLE C.3 Residue Data from Sugar Beet Field Trials with Clothianidin.

Location (City, State; Year) [Trial ID]	Zone	Variety	Matrix	Total Use Rate (lb ai/A)	Harvest (DAP)[1]	Residues (ppm)[2]		
						Clothianidin	TMG	Combined[3]
Sabin, MN 2004 [002]	5	Beta 101	Root	0.070	147	[0.006], [0.008]	ND, ND	<0.020, <0.020
			Tops			ND, ND	ND, ND	<0.020, <0.020
Theilman, MN 2004 [003]	5	Beta 101	Root	0.084	112	ND, ND	ND, ND	<0.020, <0.020
			Tops			ND, [0.004]	[0.004], [0.005]	<0.020, <0.020
Northwood, ND 2004 [004]	5	Beta 101	Root	0.079	145	0.015, [0.009]	ND, ND	<0.020, <0.020
			Tops			ND, ND	ND, ND	<0.020, <0.020
Campbell, MN 2004 [005]	5	Beta 102	Root	0.093	151	ND, [0.007]	ND, ND	<0.020, <0.020
			Tops			ND, ND	[0.005], [0.005]	<0.020, <0.020
Velva, ND 2004 [006]	7	Beta 102	Root	0.078	125	0.019, 0.015	ND, ND	<0.029, <0.025
			Tops			[0.005], [0.006]	[0.007], 0.015	<0.020, <0.025
Larned, KS 2004 [007]	8	Beta 102	Root	0.090	128	ND, [0.008]	ND, ND	<0.020, <0.020
			Tops			ND, ND	ND, [0.004]	<0.020, <0.020
Eaton, CO 2004 [008]	9	Beta 102	Root	0.088	141	ND, ND	ND, ND	<0.020, <0.020
			Tops			ND, ND	[0.006], [0.007]	<0.020, <0.020
Fresno, CA 2004 [009]	10	Beta 102	Root	0.079	179	ND, [0.005]	ND, ND	<0.020, <0.020
			Tops			0.011, 0.011	0.011, [0.007]	0.022, <0.021
Live Oak, CA 2004 [010]	10	Beta 102	Root	0.078	164	ND, ND	ND, ND	<0.020, <0.020
			Tops			ND, ND	[0.004], 0.010	<0.020, <0.020
Madras, OR 2004 [011]	11	Beta 102	Root	0.079	109	[0.006], [0.005]	ND, ND	<0.020, <0.020
			Tops			ND, ND	[0.007], [0.006]	<0.020, <0.020
Ephrata, WA 2004 [012]	11	Beta 102	Root	0.088	154	[0.004], [0.004]	ND, ND	<0.020, <0.020
			Tops			[0.008], [0.005]	0.026, 0.021	<0.036, <0.031

1. DAP = Days After Planting.
2. Residues of both analytes are expressed in clothianidin equivalents. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and tops. The statistically calculated LODs are 0.003 ppm for clothianidin in both roots and tops, and 0.001 and 0.003 ppm for TMG in roots and tops, respectively.
3. For calculating combined residues, the LOQ is used for values <LOQ.
4. Residue results in [brackets] are >LOD, but <LOQ.

TABLE C.4 Summary of Residue Data from Sugar Beet Field Trials with Clothianidin.									
Crop [Matrix]	Total Use Rate (lb ai/A)	DAP	Combined Residue Levels (ppm)[1]						
			n	Min.	Max.	HAFT[2]	Median	Mean	Std. Dev.
Clothianidin									
Sugar Beet [Root]	0.070-0.094	109-179	24	<0.010	0.019	0.017	0.010	0.011	0.002
Sugar Beet [Tops]			24	<0.010	0.011	0.011	0.010	0.010	0.000
TMG									
Sugar Beet [Root]	0.070-0.094	109-179	24	<0.010	<0.010	0.010	0.010	0.010	0.000
Sugar Beet [Tops]			24	<0.010	0.026	0.024	0.010	0.011	0.004
Combined Residues									
Sugar Beet [Root]	0.070-0.094	109-179	24	<0.020	<0.029	0.027	0.020	0.021	0.002
Sugar Beet [Tops]			24	<0.020	<0.036	0.034	0.020	0.021	0.004

1. Expressed in clothianidin equivalents. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and tops. For calculating the median, mean and standard deviation, the LOQ was used for residue values of <LOQ.
2. HAFT = Highest Average Field Trial.

D. CONCLUSION

The field trial data are adequate, and support the use of clothianidin (SE) as a seed treatment for sugar beets at a rate of 0.132 lb ai/100,000 seeds.

E. REFERENCES

PP#1F06315. TI-435 (Clothianidin) on Corn and Canola. Summary of Analytical Chemistry and Residue Data.; D282446; Yan Donovan; 5/01/2003.

Method Review - PP#1F06315. New Chemical - Clothianidin (TI-435) in/on Corn and Canola. Original Request for Petition Method Validation.; D282448; Pat Schermerhorn; 7/17/2003.

F. DOCUMENT TRACKING

RDI: W.T. Drew (5/2/2007); D. Dotson (5/17/2007)
Petition Number: 6F7159
DP Number: 335355
PC Code: 044309

Clothianidin/264-RNLA/PC Code 044309/Bayer CropScience/264
DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
Processed Food and Feed – Sugar Beet (Sugar, Dried Pulp, Molasses)

Primary Evaluator: Date: 5/4/2007

William T. Drew,
Chemist, HED/RAB2

Peer Reviewer: Date: 5/17/2007

Douglas Dotson (PhD),
Chemist, HED/RAB2

This DER was originally prepared under contract by Dynamac Corporation (1910 Sedwick Road, Building 100, Suite B, Durham NC 27713). It has been reviewed by the Health Effects Division (HED), and revised to reflect current Office of Pesticide Programs (OPP) policies.

STUDY REPORT

MRID #47007810. Francis K. Duah, Ann M. Harbin (2006) *Clothianidin + Cyfluthrin 600 SE – Magnitude of the Residue in Sugar Beet Processed Commodities.* Laboratory Project #1626. Bayer Study #RATIY007. Unpublished study prepared by Bayer CropScience. 310 pages. {OPPTS Residue Chemistry Test Guideline 860.1520}

EXECUTIVE SUMMARY

Bayer CropScience submitted a processing study supporting the use of clothianidin as a seed treatment for sugar beets. For this study, sugar beet seeds were treated with a suspo-emulsion (SE) formulation containing both clothianidin, at 4 pounds of active ingredient per gallon (lb ai/gal), and cyfluthrin (1 lb ai/gal). Cyfluthrin is not addressed in this document. The treated seeds were then planted at a field trial site in MN during 2004. Seeds were treated at a rate equivalent to 0.661 lb ai per 100,000 seeds. Based on the actual seeding rate used in the field trial, 52,900 seeds per acre (seeds/A), this rate was equivalent to 0.350 lb ai/A for clothianidin. Single bulk control and treated samples of sugar beet roots were harvested at normal commercial maturity, 147 days after planting (DAP), and placed in frozen storage. After roughly 13 months of storage, roots were processed into molasses, refined sugar, and dried pulp using simulated commercial procedures. Root samples were stored frozen for approximately 12.6 months prior to analysis, and the frozen processed commodities were analyzed within 1 month (sugar and dried pulp) or 4.3 months (molasses) of collection. These storage durations are supported by the available storage stability data.

The LC/MS/MS method (Bayer Method TI-002-P05-001) used to determine residues of clothianidin and its metabolite, TMG, in sugar beet roots and processed commodities was adequately validated in conjunction with the analysis of processing study samples. For this method, residues were extracted sequentially with acetonitrile (ACN), and ACN/water, then fortified with internal standards of [$^2H_6$]-clothianidin and [$^2H_6$]-TMG. The combined extract was acidified, and residues were purified by C_{18} solid phase extraction (SPE) prior to LC/MS/MS analysis. Residues of clothianidin and TMG are expressed in clothianidin equivalents. The validated limit of quantitation (LOQ) for both clothianidin and TMG is 0.010 ppm in sugar beet

roots and processed commodities, with the exception of clothianidin in molasses (0.020 ppm). The statistically calculated limits of detection (LODs) for clothianidin are 0.003 ppm in roots, sugar, and dried pulp, and 0.011 ppm in molasses, while the statistically calculated LODs for TMG are 0.001 ppm in roots, 0.002 ppm in sugar and dried pulp, and 0.005 ppm in molasses.

Following a seed treatment at 0.350 lb ai/A (5X exaggerated rate), residues of clothianidin averaged 0.011 ppm in roots, the raw agricultural commodity (RAC), at maturity, and averaged <0.003 ppm in refined sugar, 0.017 ppm in dried pulp, and 0.032 ppm in molasses. Residues of TMG were non-detectable in roots and all processed fractions. Processing factors for clothianidin were <0.3X in refined sugar, 1.5X in dried pulp, and 2.9X in molasses. Processing factors for combined residues were similar but slightly lower, as TMG residues were <LOD in all fractions.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

Under the conditions and parameters used in the study, the processed commodity residue data are classified as acceptable, provided that the 12-month storage stability data for TMG in potato tubers and processed fractions are submitted, and deemed adequate. The acceptability of this study for regulatory purposes is addressed in the US EPA Residue Chemistry Summary Document (DP #335355).

COMPLIANCE

Signed and dated Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an adverse impact on the validity of the study.

A. BACKGROUND INFORMATION

Clothianidin is a systemic insecticide belonging to both the chloronicotinyl (neonicotinoid) and nitroguanidine classes of chemicals. It is also a major metabolite of thiamethoxam. Clothianidin is currently registered to Bayer for use as a seed treatment on corn, canola (rapeseed), and sorghum, and to Arvesta Corporation for use as a foliar application on pome fruits, grapes, and potatoes. It enters the transpiration stream through the roots and cotyledons of newly germinating seedlings, and protects below- and above-ground plant parts from insect damage. Clothianidin binds (via ingestion and contact routes) with the nicotinic acetylcholine receptor sites, interfering with transmission of stimuli, and eventually inhibiting reproduction of the insect. Tolerances have been established for residues of clothianidin in or on plant commodities and milk at levels of 0.01-1.0 ppm (40CFR §180.586), including time-limited tolerances of 0.02 ppm in sugar beet root and tops, which expire on 12/31/2009. The residue of concern, for purposes of both risk assessment and the tolerance expression, is parent clothianidin only. However, the Agency (D282446, Yan Donovan, 5/01/2003) has indicated that for new uses on root and/or leafy vegetable crops, samples should also be analyzed for residues of the metabolite, TMG.

Bayer has submitted a petition (PP#6F7159) proposing the use of Poncho Beta (with EPA File Symbol 264-RNLA) as a seed treatment on sugar beets. The proposed product is a multiple active ingredient (MAI) formulation containing 3.33 lb/gal of clothianidin, and 0.44 lb/gal of beta-cyfluthrin, formulated as an SE. To support this use, Bayer has submitted a sugar beet processing study in which an SE formulation containing both clothianidin (4 lb ai/gal) and cyfluthrin (1 lb ai/gal) was applied as a seed treatment.

The nomenclature of clothianidin and TMG are summarized in Table A.1 (below), and the physicochemical properties of clothianidin are summarized in Table A.2 (below).

TABLE A.1 Clothianidin Nomenclature.

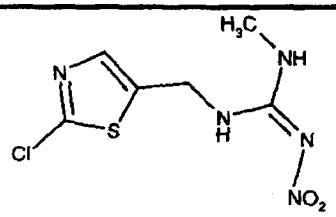
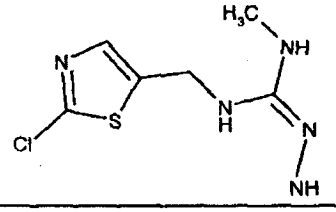
Compound	
Empirical Formula	$C_6H_8ClN_5O_2S$
Molecular Weight	249.7
Common Name	Clothianidin
Company Experimental Names	TM-444, TI-435, V-10066
IUPAC Name	*(E)*-1-(2-Chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine
CAS Name	[C*(E)*]-*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methyl-*N''*-nitroguanidine
CAS Registry Number	210880-92-5 (formerly 205510-53-8)
End-use Product (EP)	Poncho Beta (3.33 lb ai/gal SE; EPA File Symbol 264-RNLA)
Metabolite	
Common Name	TMG
IUPAC/CAS Name	*N*-[(2-Chloro-5-thiazolyl)methyl]-*N'*-methylguanidine
CAS registry Number	635283-91-9

TABLE A.2 Physicochemical Properties of Clothianidin (TGAI*).

Parameter	Value	Reference
Melting Point (°C)	176.8	MRID #45422301
pH at 23°C	6.24 (1% solution/suspension)	
Density (g/cm^3)	1.61 (PAI*), 1.59 (TGAI)	
Water Solubility (g/L) at 20°C	0.327	

TABLE A.2 Physicochemical Properties of Clothianidin (TGAI*).			
Parameter	**Value**		**Reference**
Solvent Solubility (g/L) at 25°C	Acetone	15.2	
	Dichloromethane	1.32	
	Ethyl acetate	2.03	
	Heptane	<0.00104	
	Methanol	6.26	
	Octanol	0.938	
	Xylene	0.0128	
Vapor Pressure (Pa) at 20°C	1.3×10^{-10}		
Dissociation Constant (pK_a) at 20°C	11.09		
Octanol/Water Partition Coefficient, (Log K_{OW}) at 25°C	0.7		
UV/visible Absorption Spectrum (Maximum, nm)	265.5 (acidic or neutral) 246.0 (basic)		

* PAI = Purified Active Ingredient. TGAI = Technical Grade Active Ingredient.

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

TABLE B.1.1 Study Use Pattern					
Location (County, State; Year) [Trial ID]	**End-use Product[1]**	**Application Information**			
		Method[2]; Timing	**Spray Volume**	**Rate[3] (lb ai/ 100,000 seeds)**	**Field Rate[3] (lb ai/A)[4]**
Sabin, MN 2004 [TI013-04P]	4 lb ai/gal SE	Seed treatment.	N/A	0.661	0.350

1. The SE formulation is an MAI containing 4 lb/gal of clothianidin, and 1 lb/gal of cyfluthrin.
2. Seeds were treated using a commercial Hege 11 Seed Treater.
3. Rates are for clothianidin.
4. The field application rate was calculated using the actual seeding rate (52,900 seeds/A).

B.2. Sample Handling and Processing Procedures

Single composited samples of control and treated sugar beet roots (68 kg per sample) were collected at normal commercial maturity (147 DAP) at BBCH 49. Samples were put into labeled containers, and placed in frozen storage within 1 hour of harvest. Samples were initially shipped by freezer truck to the Food Protein Research and Development Center (FPRDC) in Bryan, TX, and stored at -12°C or colder.

Approximately 11 months after collection, subsamples of the control and treated roots (RAC) were shipped by overnight courier on dry ice from FPRDC to Bayer Research Park (BRP) in Stilwell, KS, where some of the RAC samples were homogenized and stored at -15°C or colder. The RAC samples were then shipped by freezer truck to the analytical laboratory, Pyxant Labs (in Colorado Springs, CO), where they were stored at -20°C or colder until analysis.

The remaining frozen bulk root samples were transferred from FPRDC to the processing facility, GLP Technologies (in Navasota, TX), and stored at -12°C or colder. Approximately 13 months after harvest, the roots were processed into refined sugar, molasses, and dried pulp using simulated commercial processing procedures. The processed commodities were frozen after collection, and shipped within 2 weeks by overnight courier to Pyxant, where the samples were stored at -20°C or colder, and analyzed within roughly 2 weeks.

B.3. Analytical Methodology

Residues of clothianidin and TMG in sugar beet roots and processed fractions were determined using an LC/MS/MS method (Bayer Method TI-002-P05-001). This method is similar to a LC/MS/MS method (Bayer Method 00552-M001), which has been approved for enforcing tolerances on plant commodities (D282448, Pat Schermerhorn, 7/17/2003). The principal difference between these methods is that Method TI-002-P05-001 also determines the metabolite TMG.

For sugar beet roots, sugar, and dried pulp, residues were extracted sequentially with ACN (twice), and ACN/water (1:1, v/v) twice; the extracts were then combined and centrifuged. For molasses, samples were dissolved in ACN/0.03% acetic acid (40:60, v/v). For all commodities, a mixed internal standard containing [$^2H_6$]-clothianidin and [$^2H_6$]-TMG was added, and residues were diluted with 0.045% acetic acid. Residues were then purified using a C_{18} SPE cartridge, concentrated, and re-dissolved in water/methanol (9:1, v/v, each containing 0.1% formic acid). Residues of clothianidin and TMG were then determined by LC/MS/MS using the following transitions for quantitation: clothianidin, m/z 250→169; [$^2H_6$]-clothianidin, m/z 253→172; TMG, m/z 207→134; and [$^2H_6$]-TMG, m/z 210→137. The presence of clothianidin and TMG residues was confirmed using the m/z 250→132 and m/z 207→71 transitions, respectively. Residues of clothianidin and TMG are expressed in clothianidin equivalents. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and processed commodities, except molasses. The LOQs for molasses are 0.020 ppm for clothianidin and 0.010 ppm for TMG. The statistically calculated LODs for clothianidin are 0.003 ppm in roots, sugar, and dried pulp, and 0.011 ppm in molasses, while the statistically calculated LODs for TMG are 0.001 ppm in roots, 0.002 ppm in sugar and dried pulp, and 0.005 ppm in molasses.

The above method was validated in conjunction with the analysis of the processing study samples using control samples of roots, molasses, sugar, and dried pulp fortified with clothianidin at 0.010-0.050 ppm and TMG at 0.010 and 0.020 ppm.

C. RESULTS AND DISCUSSION

The LC/MS/MS method (Bayer Method TI-002-P05-001) used for determining residues of clothianidin and TMG was adequately validated in conjunction with the analysis of processing study samples. The average recovery (± standard deviation) of clothianidin was 96 ± 9% from roots, 103 ± 12% from refined sugar, 101 ± 10% from dried pulp, and 94 ± 11% from molasses (see Table C.1, below). The average recovery (± standard deviation) of TMG was 96 ± 10%

from roots, 108 ± 7% from refined sugar, 92 ± 6% from dried pulp, and 89 ± 16% from molasses. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and processed commodities, with the exception of clothianidin in molasses (0.020 ppm). The statistically calculated LODs for clothianidin are 0.003 ppm in roots, sugar, and dried pulp, and 0.011 ppm in molasses, while the statistically calculated LODs for TMG are 0.001 ppm in roots, 0.002 ppm in sugar and dried pulp, and 0.005 ppm in molasses. Apparent residues of both analytes were nondetectable in all control samples. Adequate sample calculations and example chromatograms were provided.

Samples were stored at -15°C or colder for up to 24 days (refined sugar and dried pulp), 130 days (molasses) or 385 days (roots) prior to analysis (see Table C.2, below). Storage stability data are available indicating that clothianidin is stable at -18°C for intervals of up to 24 months in sugar beet roots, and corn forage, grain and straw, and canola seeds (D282446, Yan Donovan, 5/3/2003). The study report also presented summary data from an on-going storage stability study examining the stability of TMG in potato commodities (tubers, flakes, chips), and sugar beet leaves at -15°C or colder. Although this study has not yet been reviewed by the Agency, the summary data indicate that TMG is stable in frozen potato tubers, flakes, and chips, and sugar beet leaves for up to 373 days (12 months). Provided that the TMG study is deemed adequate by the Agency, these data will adequately support the storage durations and conditions of samples in the current sugar beet processing study.

Following a seed treatment at 0.350 lb ai/A (5X rate), residues of clothianidin averaged 0.011 ppm in roots (RAC) at maturity, and averaged <0.003 ppm in sugar, 0.017 ppm in dried pulp, and 0.032 ppm in molasses (see Table C.3, below). Residues of TMG were non-detectable in roots and all processed fractions. Processing factors for clothianidin were <0.3X in refined sugar, 1.5X in dried pulp, and 2.9X in molasses. Processing factors for combined residues were similar but slightly lower, as TMG residues were <LOD in all fractions.

TABLE C.1 Summary of Concurrent Recoveries of Clothianidin and TMG from Sugar Beets (RAC) and Sugar Beet Processed Commodities.

Crop [Matrix]	Spike Level (ppm)	Sample Size (n)	Recoveries (%)	Mean ± Std. Dev. (%)
Clothianidin				
Sugar Beet [Roots]	0.010	10	89, 88, 90, 104, 109, 96, 89, 90, 102, 80	94 ± 9
	0.030	3	102, 109, 96	102 ± 7
	Total	**13**	**80-109**	**96 ±9**
Sugar Beet [Refined Sugar]	0.010	11	99, 118, 100, 114, 100, 117, 114, 108, 90, 88, 87	103±12
Sugar Beet [Dried Pulp]	0.010	9	109, 109, 86, 94, 90, 91, 89, 103, 112	98±10
	0.020	3	104, 111, 115	110±6
	Total	**12**	**86-112**	**101±10**
Sugar Beet [Molasses]	0.020	8	92, 84, 98, 110, 83, 77, 90, 92	91±10
	0.050	3	98, 107, 105	103±5
	Total	**11**	**77-110**	**94±11**

TABLE C.1 Summary of Concurrent Recoveries of Clothianidin and TMG from Sugar Beets (RAC) and Sugar Beet Processed Commodities.

Crop [Matrix]	Spike Level (ppm)	Sample Size (n)	Recoveries (%)	Mean ± Std. Dev. (%)
			TMG	
Sugar Beet [Roots]	0.010	10	88, 88, 101, 94, 93, 95, 96, 93, 95, 92	94 ± 4
	0.020	3	80, 112, 119	104 ± 21
	Total	**13**	**80-119**	**96 ± 10**
Sugar Beet [Refined Sugar]	0.010	11	112, 101, 115, 115, 117, 105, 102, 118, 104, 104, 100	108±7
Sugar Beet [Dried Pulp]	0.010	9	85, 97, 92, 93, 89, 100, 97, 82, 86	91±3
	0.020	3	92, 92, 93	92±1
	Total	**12**	**82-100**	**92±6**
Sugar Beet [Molasses]	0.010	8	79, 74, 82, 114, 86, 86, 75, 113	89±16

TABLE C.2 Summary of Storage Conditions.

Crop [Matrix]	Storage Temperature (°C)	Actual Storage Duration (Days)	Interval of Demonstrated Storage Stability (Days)
Sugar Beet [Roots]	-15	385	730 (Clothianidin) 373 (TMG)
Sugar Beet [Refined Sugar]		24	
Sugar Beet [Dried Pulp]		24	
Sugar Beet [Molasses]		130	

TABLE C.3 Residue Data from Sugar Beet Processing Study with Clothianidin.

Crop	Commodity	Total Use Rate (lb ai/A)	DAP [1]	Analyte	Residues (ppm) [2]	Processing Factor
Sugar Beet	Roots (RAC)	0.350	147	Clothianidin	0.010, 0.012, 0.010 [0.011]	NA [3]
				TMG	ND [4], ND, ND	NA
				Combined [5]	0.020, 0.022, 0.020 [0.021]	NA
	Refined Sugar			Clothianidin	ND, ND, ND [0.003] [6]	<0.3X
				TMG	ND, ND, ND	NC [7]
				Combined [5]	ND, ND, ND [0.005] [6]	<0.3X
	Dried Pulp			Clothianidin	0.015, 0.018, 0.017 [0.017]	1.5X
				TMG	ND, ND, ND	NC
				Combined [5]	0.025, 0.028, 0.027 [0.027]	1.3X
	Molasses			Clothianidin	0.029, 0.030, 0.038 [0.032]	2.9X
				TMG	ND, ND, ND	NC
				Combined [5]	0.039, 0.040, 0.048 [0.042]	2.0X

1. DAP = Days After Planting.
2. Residues of both analytes are expressed in clothianidin equivalents. Each sample was analyzed in triplicate, and average residues are in [brackets]. The validated LOQ for both clothianidin and TMG is 0.010 ppm in sugar beet roots and processed commodities, with the exception of clothianidin in molasses (0.020 ppm). The LODs for clothianidin are 0.003 ppm in roots, sugar, and dried pulp, and 0.011 ppm in molasses, while the LODs for TMG are 0.001 ppm in roots, 0.002 ppm in sugar and dried pulp, and 0.005 ppm in molasses.
3. NA = Not Applicable.
4. ND = Not Detected.

5. For calculating combined residues, the LOQ was used for residue values of <LOQ.
6. Processing factors for refined sugar were calculated using the value for the LOD.
7. NC = Not Calculated (residues of TMG were <LOD in the RAC).

D. CONCLUSION

The sugar beet processing study is adequate, provided that the supporting storage stability data on TMG in potatoes and potato processed fractions is submitted, and deemed adequate by the Agency. Following a seed treatment at 0.0350 lb ai/A (5X exaggerated treatment rate), residues in mature sugar beet roots were 0.011 ppm for clothianidin, and <LOD for TMG. Based on the clothianidin data, residues do not concentrate in refined sugar (<0.3X), but concentrated by 1.5X in dried pulp, and 2.9X in molasses. The maximum possible processing factor for sugar beet dried pulp is 20X.

E. REFERENCES

PP#1F06315. TI-435 (Clothianidin) on Corn and Canola. Summary of Analytical Chemistry and Residue Data.; D282446; Yan Donovan; 5/01/2003.

Method Review - PP#1F06315. New Chemical - Clothianidin (TI-435) in/on Corn and Canola. Original Request for Petition Method Validation.; D282448; Pat Schermerhorn; 7/17/2003.

F. DOCUMENT TRACKING

RDI: W.T. Drew (5/4/2007); D. Dotson (5/17/2007)
Petition Number: 6F7159
DP Number: 335355
PC Code: 044309

13544

R154035

Chemical: Clothianidin

PC Code:
044309

HED File Code: 14000 Risk Reviews
Memo Date: 10/16/2007
File ID: DPD335355
Accession #: 000-00-0122

HED Records Reference Center
11/1/2007